Inulin, a flexible oligosaccharide. II

Mensink, Maarten A; Frijlink, Henderik W; van der Voort Maarschalk, Kees; Hinrichs, Wouter L J

Published in:
Carbohydrate Polymers

DOI:
10.1016/j.carbpol.2015.08.022

Publication date:
2015

Carbohydrate Polymers 134 (2015) 418–428

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Inulin, a flexible oligosaccharide. II: Review of its pharmaceutical applications

Maarten A. Mensink[a], Henderik W. Frijlink[a], Kees van der Voort Maarschalk[a,b], Wouter L.J. Hinrichs[a,*]

[a] Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands
[b] Process Technology, Corbion Purac, PO Box 21, 4200 AA Gorinchem, The Netherlands

ARTICLE INFO

Article history:
Received 9 July 2015
Received in revised form 9 August 2015
Accepted 10 August 2015
Available online 13 August 2015

Keywords:
Fructan
Anhydrobiosis
Vaccines
Stabilizer
Formulation
Excipient

ABSTRACT

Inulin is a flexible oligosaccharide which has been used primarily in food for decades. Recently new applications in the pharmaceutical arena were described. In a previous review (Mensink et al. (2015). *Carbohydrate Polymers*, *130*, 405) we described the physicochemical characteristics of inulin, characteristics which make inulin a highly versatile substance. Here, we review its pharmaceutical applications. Applications of inulin that are addressed are stabilization of proteins, modified drug delivery (dissolution rate enhancement and drug targeting), and lastly physiological and disease-modifying effects of inulin. Further uses of inulin include colon specific drug administration and stabilizing and adjuvating vaccine formulations. Overall, the uses of inulin in the pharmaceutical area are very diverse and research is still continuing, particularly with chemically modified inulins. It is therefore likely that even more applications will be found for this flexible oligosaccharide.

1. Introduction

Inulin is a fructan-type oligosaccharide that can be found in a wide range of plants (Apolinário et al., 2014). It was discovered in the 19th century (Fluckiger & Hanbury, 1879) and since then its characteristics has been investigated extensively. Most commercially available inulin is extracted from chicory root, which contains a relatively high concentration of this carbohydrate (Franck, 2002). Inulin has proven to be a versatile substance with a large number of different applications mainly in food and pharma. In a previous review, we addressed the physicochemical properties of inulin that make it such a widely applicable compound (Mensink, Frijlink, van der Voort Maarschalk, & Hinrichs, 2015). Processing history and degree of polymerization have a large impact on the physicochemical behavior of inulin. As physicochemical behavior governs functionality for different applications, these characteristics should be taken into account when using inulin.

Some examples of saccharides frequently used as excipient in pharma are glucose, sucrose, trehalose, lactose, dextran and cellulose (Mensink et al., 2015). Inulin differs from these saccharides in molecular weight and/or the type of glyosidic bond between monomers. Inulin has a higher molecular weight than mono- and disaccharides, with that it has a higher glass transition and melting temperature, and is more viscous when dissolved. The higher molecular weight also correlates with a lower solubility. Compared to other oligo- and polysaccharides, inulin has a high molecular flexibility because of its $(2\rightarrow1)$ linked-*d*-fructosyl backbone. Therefore inulin has relatively low glass transition and melting temperatures compared to other oligo- and polysaccharides. These characteristics can be both advantageous and disadvantageous depending on the application at hand. Unlike most of the other mentioned saccharides, inulin is not metabolized by humans. This allows for unique applications such as determination of kidney function and colonic targeting, which makes use of metabolization by microbiota present in the colon. Reducing groups of saccharides are undesired for many pharmaceutical applications. When presence of reducing groups is a concern, inulin is more suitable as an excipient than for example glucose and lactose (Mensink et al., 2015; Tonnis et al., 2015).

Over the past decades, more and more research on food and pharmaceutical applications of inulin has been published. The approval of the Generally Recognized As Safe (GRAS) status of inulin by the United States Food and Drug Administration in 2002 seems to have provided a boost to research into applications

* Corresponding author. Tel.: +31 50 363 2398; fax: +31 50 363 2500.
E-mail address: W.L.J.Hinrichs@rug.nl (W.L.J. Hinrichs).

http://dx.doi.org/10.1016/j.carbpol.2015.08.022

of inulin (Kruger, 2002). As inulin's food applications have been reviewed before we will not discuss them here (Mensink et al., 2015). To our knowledge, only one review has been published in which pharmaceutical applications of inulin are addressed amongst other topics (Barclay, Ginic-Markovic, Cooper, & Petrovsky, 2010). In this manuscript, an overview will be given of the current pharmaceutical applications of inulin in the light of its physicochemical characteristics. Applications which will be addressed are stabilization of proteins, modified drug delivery (dissolution rate enhancement and drug targeting), and physiological and disease-modifying effects of inulin.

2. Pharmaceutical applications

2.1. Stabilization

2.1.1. Anhydrobiosis

In nature, inulin has been associated with drought protection in several plants, with increased levels of inulins found in stress resilient plants (Livingston, Hincha, & Heyer, 2007; Matvieieva et al., 2013). Membrane stabilization is believed to be an important part of the drought protection of inulin (Livingston et al., 2007). Vereyken, Albert van Kuik, Evers, Rijken, and de Kruijff (2003a), Vereyken, Chupin, Demel, Smeekens, and De Kruijff (2001), Vereyken, Chupin, Hoekstra, Smeekens, and de Kruijff (2003b) and Vereyken et al. (2003c) have published several papers on the mechanism of stabilization of fructans on membranes. They investigated the effects of inulin on model systems, such as: phospholipid monolayers, bilayers and liposomes in states varying from dry to completely hydrated. Fructans (inulin- and levan-type) were found to increase lamellar repeat distance and reduce vesicle fusion during air drying, indicating they were present between the lipid bilayers (Vereyken et al., 2003c). Fructans insert between the headgroups of several glyco- and phospholipids even if the lipid packing is very tight (Vereyken et al., 2001, 2003a,b). Fructan immobilized the lipid headgroups through direct interaction, but increased mobility of the acyl chains because the insertion of fructan caused them to be spaced further apart, in turn leading to a lower order–disorder phase transition temperature (Vereyken et al., 2003b). Additionally, fructan reduced the accessibility of the membranes, indicative of some form of coating of the membrane (Vereyken et al., 2001).

The effect of fructans on the membranes was much stronger than that of other polysaccharides such as dextran, which was explained by inulin's more hydrophobic character (Vereyken et al., 2001). Inulin showed more interaction with the headgroups than levan-type fructan (Vereyken et al., 2003a). This is probably because inulin inserts more deeply into the membrane compared to levan (Vereyken et al., 2003b). Increasing molecular weight of inulin was found to correlate with stronger interactions with the membranes (Vereyken et al., 2003a). Levan-type fructans perform worse than inulin-type fructans despite the fact that they are much larger. Apparently, some other trait of inulin must be the reason for its success. The flexibility of inulin's molecular backbone, conformation of the backbone as well as hydrophobicity were suggested as explanations for inulin's effects on these membranes (Vereyken et al., 2003a,c) Additionally, inulin's furanose groups are also smaller and more flexible compared to pyranose groups found in dextrans.

Hincha et al. also investigated membrane stabilization by oligosaccharides including inulin using liposomes as model systems (Hincha, Hellwege, Heyer, & Crowe, 2000; Hincha, Zuther, Hellwege, & Heyer, 2002; Hincha, Zuther, & Heyer, 2003; Livingston et al., 2007). During freeze-drying inulin stabilized large unilamellar vesicles of egg phosphatidylcholine, whilst hydroxyethylstarsch (HES) did not stabilize them at all. A combination of inulin and glucose was especially effective (Hincha et al., 2000). Based on findings with raffinose-family oligosaccharides, it was concluded that a higher degree of polymerization (DP) provides increased protection of liposomes against fusion at elevated temperatures and against leakage after rehydration, because of the higher glass transition temperature (T_g) of longer chain oligosaccharides (Hincha et al., 2003). A larger chain length resulted in better membrane stability during air drying when inulin-type fructans were used, but not when glucans were used (Hincha et al., 2002). Glucans were however increasingly effective at reducing membrane fusion with increasing chain length, contrary to inulin (Hincha et al., 2002). The limited solubility of larger inulin (DP > 10) caused it to precipitate during the used slow air-drying and prevented it from stabilizing the liposomes (Livingston et al., 2007). In agreement with the findings of Vereyken et al. discussed above, Hincha et al. (2000, 2002) also found a direct interaction of inulin with the phospholipid in the dry state and a reduction of the gel to liquid-crystalline phase transition temperature.

Livingston et al. (2007) have provided an in-depth review on abiotic stress tolerance in plants by fructans. It shortly describes current, more general theories on how (oligo)saccharides stabilize membranes, mainly the vitrification theory and the water replacement theory. The water replacement theory explains how sugars stabilize membranes by replacing the hydrogen bonds of water with the membrane during drying and thereby inserting into the lipid bilayer (Vereyken et al., 2003b). Overall, it seems that inulin's characteristics, the structural flexibility of the backbone and furanose groups, conformation, and hydrophobic-hydrophilic balance allow it to interact with the membranes better than other oligosaccharides as described above. Inulin's flexibility in particular allows it to overcome the steric hindrance which usually inhibits sugar–membrane interactions (Livingston et al., 2007; Vereyken et al., 2003c).

The vitrification theory states that sugars form glasses instead of crystals during drying. The viscosity of a glass is extremely high, resulting in practically no diffusion and restricted molecular mobility of the membranes. A kinetically stable glass is the result. The glass should remain amorphous, as crystallization would lead to a loss of protein–sugar interactions. Clearly, a higher T_g then correlates with better stabilization (Livingston et al., 2007). Therefore, vitrification alone cannot explain the stabilizing effect of fructan (Crowe, Carpenter, & Crowe, 1998; Crowe, Leslie, & Crowe, 1994; Crowe, Oliver, Hoekstra, & Crowe, 1997), as other oligosaccharides with even higher T_gs, such as dextran and hydroxyethyl starch, did not provide better stabilization. Both vitrification and water replacement are needed for stabilization (Crowe et al., 1998). Inulin is unique in its ability to vitrify as an oligosaccharide, whilst maintaining a good interaction with the membrane as if it was a smaller saccharide, thus combining requirements for both the vitrification and water replacement theories.

2.1.2. Protein stabilization

The mechanisms behind membrane stabilization in nature and protein stabilization in the pharmaceutical arena are very similar, with the vitrification theory and water-replacement theory also being applicable to protein stabilization (Crowe et al., 1998; Crowe, Hoekstra, & Crowe, 1992). Recently, the role of these two different mechanisms under different conditions was investigated (Grasmeijer, Stankovic, de Waard, Frijlink, & Hinrichs, 2013). It was concluded that if the T_g of a sample is at least 10–20 °C above storage temperature, water replacement is the predominant mechanism of stabilization. However if the T_g is lower, vitrification limits stability (Grasmeijer et al., 2013). In other words, if the T_g is high enough to immobilize the protein, the amount of interaction between the sugar and the protein determines its stability, yet if the T_g is too low, the (lack of) immobilization becomes the limiting factor.

Other physico-chemical characteristics of sugars required for good stabilization are low hygroscopicity, low crystallization rate and little or no reducing groups. For freeze-drying a high T_g of the maximum freeze-concentrated solution (T_g') is also desired. Compared to trehalose, which is a frequently used protein stabilizer, inulin has similar hygroscopicity, it crystalizes less rapidly and the longer chain inulins have higher T_g and T_g' values (Mensink et al., 2015). Additionally, higher molecular weight inulin has fewer reducing groups, a higher glass transition temperature and with that a lower tendency to crystallize for example when exposed to ambient moisture. This being given, it is not surprising that inulin was shown to be a good stabilizer of proteins under various conditions and stresses. Inulin has been successfully used to stabilize proteins during spray-drying (Grasmeijer et al., 2013; Haj-Ahmad, Elkordy, Chaw, & Moore, 2013; Saluja et al., 2010), freeze-drying (lyophilization) (Amorij et al., 2007b; Hinrichs, Prinsen, & Frijlink, 2001; Rodríguez Furlán, Lecot, Pérez Padilla, Campderrós, & Zaritzky, 2011; Rodríguez Furlán, Padilla, & Campderrós, 2010; Rodriguez Furlán, Pérez Padilla, & Campderros, 2011; Tonnis et al., 2015) and spray-freeze drying (Amorij et al., 2007b; Saluja et al., 2010; Wahjudi et al., 2013). However, inulin does have some reducing groups, limiting its applicability to some extent (Zijlstra et al., 2009a). The number of reducing groups is dependent on processing and DP and is generally limited (Mensink et al., 2015).

Inulin, trehalose and dextran have been identified as suitable cryo- and lyoprotectants for the major constituent of influenza subunit vaccine, i.e. haemagglutinin (HA), meaning they all stabilized HA during freezing and freeze-drying (Amorij et al., 2007a). During storage of 4 different model proteins after lyophilization, inulin was a better protein stabilizer than a similar-sized dextran (Tonnis et al., 2015). Storage temperature was well below the T_g of the formulations to allow for optimal vitrification. Water-replacement was therefore considered to be dominating protein stability. It was suggested that inulin encountered less steric hindrance than dextran when interacting with the protein, because of inulin's higher molecular flexibility. Lyophilization of alkaline phosphatase without stabilizer reduced its activity to around 5%, but when either inulin, trehalose or glucose was added, full activity was maintained (Hinrichs et al., 2001). When added to bovine plasma protein, inulin also reduced denaturation during freeze-drying similar to sucrose and glucose, with an optimal concentration of 10% w/v (Rodríguez Furlán et al., 2010, 2011).

During spray-freeze-drying, spraying a solution into liquid nitrogen followed by freeze-drying, HA stabilized with inulin in hepes buffer was not structurally altered and it did not lose its antigenetic properties (Amorij et al., 2007b; Saluja et al., 2010). When PvdQ, acyl-homoserine-lactone (AHL) acylase, was spray-freeze-dried, mannitol, trehalose and inulin all fully maintained its activity (Wahjudi et al., 2013). Inulin has also been applied as a protein stabilizer during hot-melt extrusion, in which a polymer based product for the controlled release of protein is created. As lysozyme is a relatively thermostable protein (Pfeil & Privalov, 1976), no degradation was found during extrusion at 55 °C. It was however suggested that for a more labile protein, inulin could also provide protection to the heat stress induced by this process (Stanković et al., 2013).

The main goal of the various processes described above is to achieve a product that is stable over time. Table 1 provides an overview of reports on inulin's stabilizing effect on dried proteins during storage under various conditions. Tonnis et al. (2015) reported degradation kinetics of their 4 model proteins during storage (60 °C, <10% RH, 28 days), but not the remaining activity after storage. Their results are therefore not included in this table, the reader is directed to that article for further information.

At room temperature inulin proved to be an equally good or better stabilizer than other saccharides. With increasing relative humidity, inulins with a higher DP perform better. This is likely because those inulins have a higher intrinsic glass transition temperature and can thus absorb more water before this lowers the T_g of the mixture to below the storage temperature. The relatively small inulin with DP 6, also called oligofructose, caused a loss of activity of around 50% in alkaline phosphatase. Degradation that may be induced by the relatively high amount of reducing groups in that particular inulin sample. This level of degradation was not found with the other, larger inulins tested (Hinrichs et al., 2001). When lyophilized alkaline phosphatase formulations with trehalose were compressed into tablets, the very brief exposure to atmospheric moisture and compacting forces resulted in formation of trehalose anhydrate crystals, leading to a complete loss of activity of the incorporated protein within 1 week of storage at 60 °C/0%RH. In contrast, inulin remained amorphous and maintained 75% activity of the protein after 3 months of storage at 60 °C/0%RH. In fact, inulin did not even crystallize when stored at 60 °C, 33% RH for two days (Eriksson et al., 2002). PvdQ spray freeze dried with mannitol stored at 55 °C also resulted in crystallization of the sugar, leading to a complete loss of activity (Wahjudi et al., 2013). Inulin and dextran were unable to maintain activity of air-dried samples of restriction enzyme PstI stored at 37 °C (Colaço, Sen, Thangavelu, Pinder, & Roser, 1992). It is most likely that limited solubility of inulin caused it to precipitate during this slow drying process, in turn preventing it from stabilizing the protein as was also seen with air-dried liposomes (Livingston et al., 2007). Unfortunately, the authors did not report the DP of the inulin, which influences its solubility (Colaço et al., 1992).

At elevated temperatures, the stabilizing capacities of different saccharides varies. As mentioned above, if vitrification is sufficiently achieved, the stabilizer with most interactions with the protein is likely to stabilize best. Oligosaccharides, however, are more sterically hindered than smaller mono- or disaccharides, reducing their interactions with the protein (Tonnis et al., 2015). Inulin's molecular flexibility is able to compensate this to some extent, unlike the more molecularly rigid dextran (Amorij et al., 2007a; Tonnis et al., 2015). Thus depending on the storage temperature and the T_g of the sample, a longer chain length of the oligosaccharide (resulting in a higher T_g) could be beneficial if vitrification is lacking, yet it could also have the opposite effect if more protein–sugar interaction is needed. As mentioned inulin has a small amount of reducing groups, meaning some protein degradation because of the Maillard reaction might occur. Yet even under extreme storage conditions (85 °C/0%RH/42days) this only played a minor role in protein stability of rhDNase, a protein that is sensitive to this type of degradation (Zijlstra et al., 2009a). In summary, inulin is a good stabilizer of proteins in the dry state, and depending on the formulation and storage conditions it can be better than smaller sugars.

2.1.3. Other stabilization

Apart from membranes and proteins, inulin has also been used to stabilize several pharmaceutically relevant systems. For example, (PEGylated) liposomes, polyethylenimine based polyplexes, lipoplexes (Hinrichs et al., 2006; Hinrichs, Sanders, De Smedt, Demeester, & Frijlink, 2005), polymersomes (Ayen & Kumar, 2012), influenza virosomes with and without encapsulated plasmid DNA (De Jonge et al., 2007), whole inactivated influenza virus (Audouy et al., 2011), recombinant adenovirus (Chen et al., 2012), and Δ^9-tetrahydrocannbinol (THC) (Van Drooge et al., 2005; Van Drooge et al., 2004b).

This variety of applications shows that under the appropriate conditions inulin may provide stabilization against chemical degradation (e.g. THC) as well as against physical degradation

Table 1
Overview of reported storage stabilities of proteins dried with inulin and other sugars. AP = alkaline phosphatase, DNase = recombinant human DNase, HA = haemagglutinin, Lys = lysozyme, PstI = restriction enzyme PstI, PvdQ = acyl-homoserine lactone acylase PvdQ; AD = air dried, FD = freeze-drying, SD = spray-drying, SFD = spray-freeze-drying, T = tableting, C = crystallization. n.r. = not reported.

Protein	Protein:sugar ratio (w/w)	Preparation	Storage conditions			Activity/potency loss after storage		Article cited
			T (°C)	RH (%)	t (days)	Stabilizer	Loss*	
PvdQ	1:100	SFD	20	<10%	28	Inulin DPn 23; trehalose	None	Wahjudi et al. (2013)
						Mannitol	Some	
						Liquid control (after 7 days)	None	
AP	1:9	FD	20	0	28	Inulin DPn 6**,14,23	None	Hinrichs et al. (2001)
						Glucose, trehalose	None	
						No stabilizer	Complete	
AP	1:19	FD	20	0	105	Inulin DPn 23, trehalose	None	Eriksson et al. (2002)
HA	1:47	FD	20	0	182	Inulin DPn 6, DPn 14	<20%	Amorij et al. (2007a)
						Trehalose, dextran56kDa	<20%	
						No stabilizer	Complete	
HA	1:200	SD,SFD	20	10	1085	Inulin DPn 23	None	Saluja et al. (2010)
						Liquid control 4 °C	Complete	
AP	1:9	FD	20	45	28	Inulin DPn 14,23; trehalose	No	Hinrichs et al. (2001)
						Inulin DPn 6	~25%	
						Glucose	~50%	
						No stabilizer	Complete	
AP	1:9	FD	20	60	28	Inulin DPn 14,23; trehalose	None	Hinrichs et al. (2001)
						inulin DPn 6; glucose	~50%	
						No stabilizer	Complete	
PstI	n.r.	AD	37	n.r.	7	Trehalose	None	Colaço et al. (1992)
						Maltotriose	80–90%	
						Inulin; dextran ***	Complete	
HA	1:47	FD	45	11	28	Inulin DPn 14	38%	Amorij et al. (2007a)
						Inulin DPn 6	24%	
						Trehalose	23%	
						Dextran 56kDa	65%	
						No stabilizer	89%	
HA	1:47	FD	45	11	182	Inulin DPn 6,14; dextran 56kDa; no stabilizer	Complete	Amorij et al. (2007a)
						Trehalose	20%	
PvdQ	1:100	SFD	55	<10%	28	Inulin DPn 23;trehalose	No	Wahjudi et al. (2013)
						Mannitol	Complete	
						Liquid control (after 7 days)	Complete	
AP	1:9	FD	60	0	6	Inulin DPn 14,23	~50%	Hinrichs et al. (2001)
						Inulin DPn 6; trehalose; glucose; no protectant	Complete	
AP	1:1–1:20	SD	60	≤6%	19	Inulin DPn 23	30%	Grasmeijer et al. (2013)
						Trehalose	10%	
DNAse	1:11	FD	60	0	42	Sucrose; trehalose; inulin DPn 14, 23	~20%	Zijlstra et al. (2009a)
						No protectant	~65%	
AP	1:19	FD	60	0	90	Inulin DPn 23	20%	Eriksson et al. (2002)
						Trehalose	None	
AP	1:19	FD+T	60	0	105	Inulin DPn 23	25%	Eriksson et al. (2002)
						Trehalose	Complete	
DNAse	1:11	FD	85	0	42	Trehalose	~50%	Zijlstra et al. (2009a)
						Inulin DPn 14, DPn 23	~70%	
						Sucrose; protectant	Complete	

* If loss is listed as none, no significant loss was detected.
** Inulin DPn 6 can also be referred to as oligofructose.
*** Degree of polymerization/molecular weight was not reported.

(e.g. lipoplexes). Inulin fully protected PEGylated lipoplexes against aggregation during lyophilization and 3 months of storage thereafter, whilst dextran did not (Hinrichs et al., 2005). Inulins were also preferred as stabilizer over dextran for various other types of PEGylated nanoparticles due to their compatibility with PEG (Hinrichs et al., 2006).

In preserving physicochemical characteristics of doxorubicin-loaded $(PEG)_3$-PLA nanopolymersomes during lyophilization,

inulin was found to be superior over trehalose, sucrose, mannitol, lactose, polyvinylpyrrolidone, and several other excipients (Ayen & Kumar, 2012). Inulin also fully preserved influenza virosomes during lyophilization. Compared to a liquid dispersion, the powder had substantially prolonged preservation of potency and in case of influenza virosomes with encapsulated plasmid DNA transfection activity (De Jonge et al., 2007). A combination of mannitol and inulin improved the therapeutic applicability of a recombinant adenovirus during freeze-drying and storage (Chen et al., 2012). Lastly, incorporation of THC in an inulin matrix by lyophilization strongly increased its stability (Van Drooge et al., 2004b). Spray-freeze drying THC with inulin turned out to provide even better stabilization than freeze-drying, likely because of the much faster cooling rate (Van Drooge et al., 2005). Inulin might therefore also be an interesting stabilizer for other unstable drugs.

2.2. Drug delivery

2.2.1. Solution behavior alteration

2.2.1.1. Solution rate enhancement. For oral drug administration, drugs need to be dissolved before they can be absorbed by the intestinal membrane. Therefore, the bioavailability of drugs with low aqueous solubility (Biopharmaceutics Classification System class II and class IV drugs) is usually poor because they slowly dissolve in the gastro-intestinal track. One of the strategies that can be applied to increase the dissolution rate is the production of a solid dispersion that is composed of a hydrophilic carrier in which the drug is finely dispersed. When this soluble carrier dissolves rapidly, the poorly soluble drug is hydrated faster, resulting in faster dissolution. Inulin has been used to produce solid dispersions of various poorly soluble drugs (Srinarong, Faber, Visser, Hinrichs, & Frijlink, 2009; van Drooge, Hinrichs, & Frijlink, 2004a; Van Drooge et al., 2004b; Visser et al., 2010). Solubility of inulin is much better than these drugs and is dependent on the molecular weight of inulin, with smaller inulins having a higher solubility (Wada, Sugatani, Terada, Ohguchi, & Miwa, 2005).

Dissolution behavior of solid dispersion tablets made of diazepam in inulin was significantly better than those based on dispersions with disaccharides like sucrose or trehalose (Van Drooge et al., 2004a). It was found that the dissolution rate of the disaccharides was extremely high, resulting in supersaturation of diazepam in the near environment of the dissolving tablet, causing it to subsequently precipitate into large crystals with obviously detrimental consequences on the release profile. This uncontrolled crystallization did not occur for inulin included diazepam, due to its lower dissolution rate (Van Drooge et al., 2004a). Fig. 1 illustrates the difference in release profiles of the sugars and diazepam for trehalose and sucrose formulations, and the similar release profiles for the solid dispersions of inulin and diazepam.

However, using fenofibrate as model drug, the dissolution behavior of the inulin based solid dispersion tablets at high drug load (50 wt%) was poor, probably also due to recrystallization of the incorporated drug during dissolution. This disadvantage could be circumvented by the incorporation of superdisintegrants, croscarmellose sodium or sodium starch glycolate, in solid dispersion (Srinarong et al., 2009). Similar solid dispersion tablets based on polyethylene glycol 20K and and polyvinylpyrrolidone K30 also exhibited excellent dissolution behavior, but were outperformed by those based on inulin after they were stored for 3 months at 20 °C/45% RH or 40 °C/75% RH (Srinarong et al., 2009). Release of TMC240, a HIV protease inhibitor, was improved both in vitro and in vivo after oral administration due to incorporation in a solid dispersion of inulin (Visser et al., 2010). Lyophilization of nifedipine with relatively low concentrations (up to 10%) of inulin directly in gelatin capsules increased dissolution rate, but as dissolution measurements were not carried out under sink conditions the overall dissolution enhancement was limited (Crum, Elkordy, Zarara, & Elkordy, 2013).

Inulin improved the dissolution of irbesartan in a physical mixture, with gradually increasing dissolution rates with increasing inulin content. Chemically modified poly(acrylic acid) grafted inulin was less successful at increasing dissolution behavior for irbesartan (Fares, Salem, & Khanfar, 2011). Another chemically modified form of inulin tested for dissolution rate enhancement is Inutec SP1. It is an inulin with alkyl groups grafted on primary or secondary hydroxyl groups of the polyfructose backbone and acts as a polymeric surfactant (Janssens, Van Humbeeck, & Van den Mooter, 2008; Van den Mooter, Vervoort, & Kinget, 2003). Itraconazol was incorporated in a solid dispersion of Inutec SP1 via spray drying and hot-stage extrusion. Even though phase separation was found and part of the itraconazol was crystalline, the dissolution behavior of the solid dispersion was better than that of itraconazol alone (Van den Mooter et al., 2003). It was later shown that spray dried itraconazol and Inutec SP1 showed no interaction and addition of an itraconazol compatible polymer was needed to obtain a molecular dispersion (Janssens et al., 2008). Contrastingly, when solid dispersions with high drug loads were produced by spray freeze-drying, Inutec SP1 was found to have superior dissolution behavior compared to inulin and polyvinylpyrrolidone (Srinarong et al., 2011).

Amorphous unmodified inulin has been shown to be a suitable filler-binder for tablet production, with chain length dependent disintegration time (Eissens, Bolhuis, Hinrichs, & Frijlink, 2002). It was also possible to produce tablets from freeze-dried inulin formulations, in which case preconditioning of the inulin powder at 45% RH prior to tableting produced the best tablets (Eriksson et al., 2002). It was also possible to make a tablet for sublingual administration of poorly soluble THC based on a solid dispersion with inulin (Van Drooge et al., 2004b). For regular delivery to the GI tract, dissolution of 80% of the drug in around 20–30 min is normal, whereas sublingual formulations require a dissolution time of a few minutes at the most. The inulin based sublingual formulation showed complete release within around 3 min.

2.2.1.2. Modified release. Inulin could be used to alter the release profile of lysozyme from a hydrophilic multiblock copolymer aimed at prolonged release, because inulin acted as a pore-former (Stanković et al., 2013). Some studies have also been published in which drugs are encapsulated with inulin or chemically modified inulin for prolonged release (Poulain et al., 2003; Wu & Lee, 2000). Acetylation of inulin strongly reduces its solubility (in a buffer of pH 7.4). Additional succinylation can then be used to increase solubilty, allowing control over solubility and with that the release profile of microspheres from this material (Wu & Lee, 2000). Microspheres of this modified inulin produced by solvent precipitation showed a porous interior and different particle size distributions for the different drugs used (Wu & Lee, 2000). Chymotrypsin containing microspheres, with a particle size distribution from 0.5 to 4 μm, showed release for up to a week with a release of nearly 70% at the first time point of 24 h. Chlorhexidine containing particles were significantly larger, i.e. 90–130 μm, and showed a similar burst release, followed by a release rate that was slower than the chymotrypsin microspheres (Wu & Lee, 2000). Overall, the prolonged release is limited, since most of the drug is released in the initial burst.

Inulin, inulin acetate (inac) and inulin acetate associated with 1,12-dodecanedicarboxylic acid (inac-dia) were used in the encapsulation of serine protease inhibitors using coacervation, producing particles with a size ranging from 0.5 to 5 μm (Poulain et al., 2003). Inulin and inac microspheres showed a burst release of around

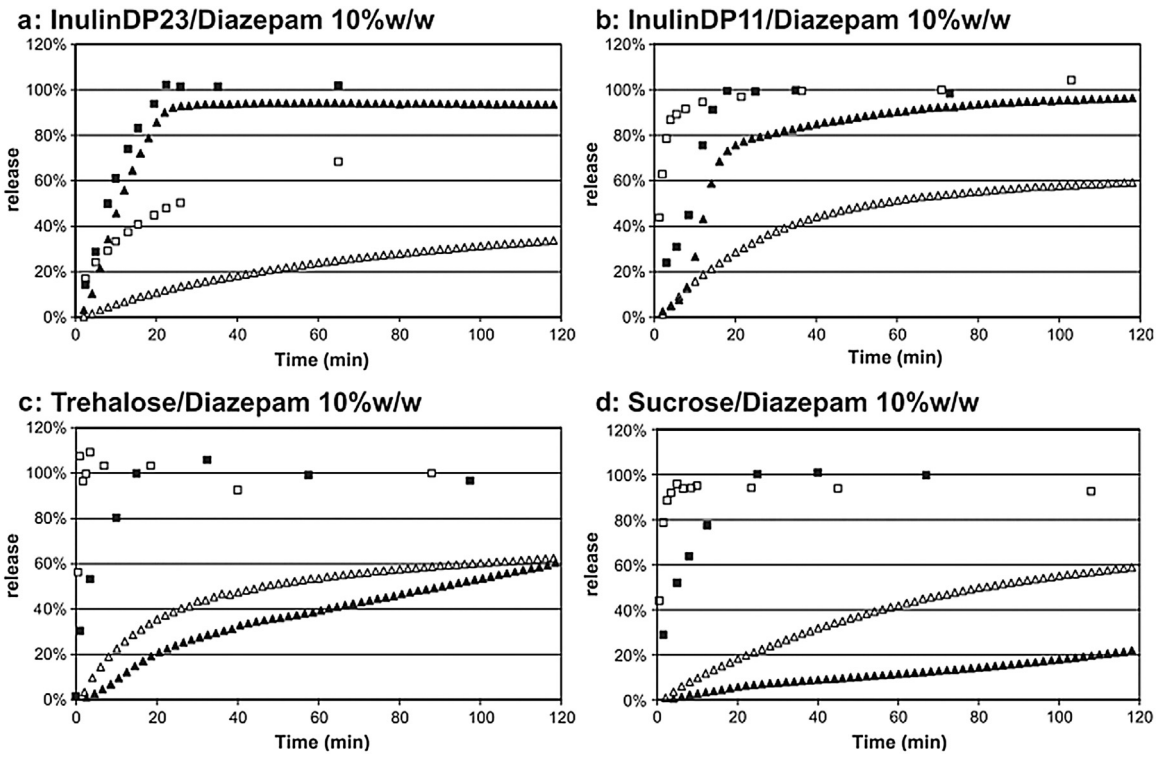

Fig. 1. Dissolution of tablets prepared from solid dispersions and physical mixtures containing 10% w/w diazepam prepared with different sugars: (a) inulin DP23, (b) inulin DP11, (c) trehalose, (d) sucrose. (Key: ■: sugar from solid dispersion, ▲: diazepam from solid dispersion □: sugar from physical mixture, △: diazepam from physical mixture) ($n = 3$ tablets, all s.d. < 10%) (Van Drooge et al., 2004a).

60% in the first minutes of dissolution, with a controlled release of the rest of the dose over a period of 4 days. Inac-dia displayed an initial burst release of only 32% after 15 min, but showed an additional burst release of around 30% after about 1.5 days due to erosion, followed by prolonged release similar to the inuline and inac spheres (Poulain et al., 2003). Another example of controlled release with a chemically modified inulin is the chemical coupling of inulin to ibuprofen using *N*,*N*′-cabonyldiimadazole. Nanoparticles consisting of methylprednisolone and this ibuprofen-inulin could be produced, providing an interesting combination therapy for spinal cord injury (Zhang et al., 2014). Lastly, it was recently shown that inulin conjugated with diethylenetriamine might be suitable as a carrier for therapeutic delivery of small interfering RNAs (Sardo et al., 2015).

2.2.2. Local drug delivery

2.2.2.1. Colon targeting. Chemically modified inulin was also applied in controlled delivery to the colon. The mechanism behind colon targeting with inulin is based on the fact that inulin is only significantly hydrolyzed by inulinases produced by bifidobacteria in the colon and not by the digestive enzymes in the upper parts of the gastro intestinal tract (Flamm, Glinsmann, Kritchevsky, Prosky, & Roberfroid, 2001; Roberfroid & Delzenne, 1998; Van den Mooter et al., 2003). This means that gels and coatings of inulin are not hydrolyzed until these reach the colon where they are fermented, resulting in a colon-specific release of incorporated drug. Several reports have described chemically modified methacrylated inulin (IN-MA) for colonic targeting (Vervoort et al., 1997; Vervoort, Rombaut, Van den Mooter, Augustijns, & Kinget, 1998). These modified inulin chains can be covalently cross-linked to each other and optionally in combination with other monomers using free-radical polymerization resulting in the chemical formation of a gel (Maris et al., 2001).

Hydrogel characteristics such as swelling need to be well chosen for controlled release. If the gel swells too much, premature release will occur. On the other hand to achieve a fast enough release in the colon, sufficient swelling is needed to allow for inulinase to access and hydrolyze the inulin (Maris et al., 2001; Vervoort et al., 1998). Finding this balance has been proven difficult, but not impossible (Maris et al., 2001). Gels which were co-polymerized with *N*,*N*′-bis(methacryloylamino)azobenzene could be degraded both through breakdown by inulinase as well as reduction of the introduced azo group by bacterial strains present in the colon (Stubbe, Maris, Van den Mooter, De Smedt, & Demeester, 2001). The molecular weight of inulin used is also relevant here, as higher molecular weight inulins form stronger gels and have higher viscosity, in turn influencing their hydrogel characteristics (Mensink et al., 2015).

IN-MA was used for the colonic delivery of bovine serum albumin, with release being mainly dependent on degree of substitution (DS, defined as the amount of methacryloyl groups per 100 fructose units) of inulin and concentration of IN-MA used (Van den Mooter et al., 2003; Vervoort et al., 1998). Two formulations, one with 27% w/w IN-MA and a DS of 8.1 and another with 22% w/w IN-MA and a DS of 12.1 were most promising for colonic delivery (Van den Mooter et al., 2003). Apart from varying DS and concentration of polymer, copolymerization with other monomers, either more hydrophobic or more hydrophilic, could also modify release profiles (Maris et al., 2001). Another approach was to use a two-step chemical modification of inulin with first methacrylic anhydride (MA), followed by succinic anhydride (SA) for a pH-sensitive release. Hydrogels of inuline-MA-SA showed a pH-responsive delivery of ibuprofen and diflunisal (Castelli et al., 2008; Tripodo, Pitarresi, Palumbo, Craparo, & Giammona, 2005). It was also possible to incorporate IgG during the formation of an inuline-MA_1-SA hydrogel, achieving controlled colonic release of the protein (Tripodo, Pitarresi, Cavallaro, Palumbo, & Giammona, 2009).

Also several other attempts have been made to combine the metabolic degradation dependent release of inulin with a pH sensitive release, but without chemical modification and crosslinking. Free films of inulin with several varieties of Eudragit® were produced and evaluated for colonic delivery. Combinations of inulins with Eudragit RS and RL had more potential for colonic delivery than combinations of inulin and other Eudragit types (Akhgari, Farahmand, Afrasiabi Garekani, Sadeghi, & Vandamme, 2006). An example of a tablet formulation for the delivery of aceclofenac to the colon that has been shown to be successful in an in vivo study is based on a combination of the pH independent Eudragit® RS100 and inulin (Sharma & Pathak, 2013).

Recently some non-gel colonic delivery systems based on modified inulin have been described. Inulin chemically bonded with cinnamate was used to form drug containing vesicles for colonic targeting. The cytostatic methotrexate was successfully encapsulated in these inulin cinnamate vesicles. However, this system showed a methotrexate release of 30–40% in 24 h even in the absence of inulinase (López-Molina et al., 2015). This is undesirable for a cytostatic agent. Another way to produce inulin particles for colonic targeting is by electrospraying inulin. To make inulin suitable for electrospraying it was first acetylated. Particles containing indomethacin produced using this method showed colonic-specific release (by inulinase) in vitro (Jain, Sood, Bora, Vasita, & Katti, 2014).

2.2.2.2. Pulmonary delivery. Pulmonary administration of drugs is often desirable for pulmonary diseases and it can also be a good route for systemic drug administration. The aerodynamic particle size (APS) of the inhaled aerosol is of great importance for pulmonary delivery. An APS of 1–5 μm is considered essential to obtain an adequate lung deposition (Amorij et al., 2007b). Both spray drying and spray-freeze drying can be used to produce particles with a broad APS distribution, allowing targeting of the airways. As mentioned in Section 2.1.2, inulin containing formulations have been used to stabilize a vaccine and other proteins during spray drying and spray-freeze drying.

A spray-freeze dried influenza subunit vaccine stabilized by inulin was suitable for inhalation and induced systemic, mucosal, humoral and cell-mediated immune responses in mice after pulmonary administration (Amorij et al., 2007b). Inhaled spray dried and spray-freeze dried influenza subunit vaccines formulations induced higher serum IgG titers than intramuscular administration (Saluja et al., 2010). Influenza whole inactivated virus vaccine spray freeze-dried with inulin was also safe and efficient for pulmonary immunization (Audouy et al., 2011). Combined with the improved storage stability pulmonary administration of inulin stabilized dry powder influenza vaccines seems promising (Audouy et al., 2011; Saluja et al., 2010). Inulin is thus a suitable stabilizer for dry powder formulations of vaccines for pulmonary administration (Tonnis, Lexmond, Frijlink, de Boer, & Hinrichs, 2013).

Spray freeze-drying of acyl-homoserine lactone acylase PvdQ, an enzyme that can be used in the treatment of *Pseudomonas aeruginosa* infections, with either trehalose or inulin produced stable formulations. The powder particles had an average aerodynamic diameter of ~1.8 μm, indicating they would be suitable for inhalation and might be used in the treatment of chronic infections in cystic fibrosis patients (Wahjudi et al., 2013). Another example of a therapeutic spray-freeze dried with inulin is cyclosporine A, which might be used to prevent allograft rejection for lung transplant patients (Zijlstra et al., 2009b). Using spray-drying, an inulin-based formulation of rhDNase was produced with a mass mean aerodynamic diameter of 2.3 μm, making it a suitable therapeutic for patients with cystic fibrosis (Zijlstra et al., 2009a). Lastly, inulin-stabilized spray freeze-dried particles containing THC were also suitable for inhalation (Van Drooge et al., 2005).

2.3. Physiological and disease-modifying effects

2.3.1. Systemic

2.3.1.1. Vaccine adjuvant. Apart from being a suitable stabilizer of vaccines, several papers have reported an adjuvant role of inulin in obtaining an immune response upon vaccination. Mostly the crystalline types of inulin, γ- and δ-inulin, which are virtually insoluble at 37 °C, were used. These crystalline forms consist of inulin with a relatively high molecular weight, the more soluble α and β forms also contain lower molecular weight inulin fractions (Cooper & Carter, 1986a). More information on the different subtypes of crystalline inulin can be found in the first part of this review (Mensink et al., 2015). It was shown that γ-inulin enhances both humoral and cell-mediated immune responses in various animal models for a wide variety of antigens, making it a very interesting adjuvant for vaccines (Cooper & Steele, 1988; Silva, Cooper, & Petrovsky, 2004). Cooper and Carter (1986b) reported γ-inulin specifically activates the alternative complement pathway, whereas the more soluble isoforms of inulin, α and β, were found to be biologically inactive and even hindered pathway activation by γ-inulin. The δ-inulin isoform had not been discovered at that time, but most likely acts as an adjuvant according to the same mechanism as γ-inulin.

It was found that δ-inulin was more immunoactive than γ-inulin (Cooper & Petrovsky, 2011). Thus far, it has for example been applied to enhance the potency of a trivalent human seasonal influence vaccine in mice (Honda-Okubo, Saade, & Petrovsky, 2012), split-virion H5N1 influenza in ferrets (Layton et al., 2011), a pulmonary formulation of whole inactivated H1N1 influenza vaccine in mice (Murugappan, Frijlink, Petrovsky, & Hinrichs, 2015) and hepatitis B in mice, guinea pigs and humans (Gordon, Kelley, Heinzel, Cooper, & Petrovsky, 2014; Saade, Honda-Okubo, Trec, & Petrovsky, 2013). It was also shown that co-administration of δ-inulin with inactivated H1N1 influenza vaccine achieved a better immune response in pregnant mice, both for the mother and the pups (Honda-Okubo, Kolpe, Li, & Petrovsky, 2014).

In contrast to the above, Kumar and Tummala (2013) have reported that it is possible to use soluble inulin for both vaccine stabilization and as an adjuvant. They developed soluble inulin microparticles which they loaded with ovalbumin and achieved a robust immune response, outperforming antigens adjuvated by traditionally used aluminum salts. It would be interesting to see further research to confirm that soluble inulin can also be an adjuvant and if confirmed, further investigation into the mechanism behind turning soluble inulin into an adjuvant.

2.3.1.2. Kidney function. A widely established application of inulin is as a diagnostic for kidney function testing, for this application inulin is intraveneously injected. Inulin is highly suitable for this application because it is distributed over the extracellular volume only and it is not metabolized. Furthermore it is only excreted via glomerular filtratrion and not resorbed by renal tubules. This makes it the most accurate substance for determination of glomerular filtration rate (The Editors of Encyclopaedia Brittanica, 2015). The test can be executed by administering inulin as a bolus or constant infusion and measured concentrations of inulin in both urine and plasma can be used for the determination of the filtration rate (Orlando, Floreani, Padrini, & Palatini, 2002). Because inulin is filtered out freely because it is relatively small, the excretion rate is directly proportional to the glomerular filtration rate (The Editors of Encyclopaedia Brittanica, 2015). As the kidneys do resorb water, the ratio between the concentrations of inulin in the tubular fluid plasma can be used to determine water resorption in the kidney (Feher, 2012). However, a drawback of this method is the detection of inulin in biological matrices (The Editors of Encyclopaedia Brittanica, 2015). Several analytical techniques have

been described in literature, but care should be taken in comparing results from different analytical methods as differences are not always negligible (Delanaye et al., 2012).

2.3.2. Gastro-Intestinal tract

2.3.2.1. Constipation. Inulin is widely used as a dietary fiber and prebiotic in so-called functional foods. These uses are worth mentioning in this context, but are not pharmaceutical applications and we will thus suffice by referring to some reviews on this topic. Flamm et al. (2001) reviewed inulin as a dietary fiber, Kolida, Tuohy, and Gibson (2002) made a comprehensive overview of the prebiotic effects of inulin and oligofructose and lastly Kelly (2008, 2009) produced an extensive two-part review about inulin-type prebiotics also in relation to some physiological effects. Nutrition can be used to provide health benefits by modification of gut microbial flora (Flint, Scott, Louis, & Duncan, 2012). In fact, inulin's effect on gut flora and gut mobility has been linked to a variety of beneficial effects, both local and systemic (Meyer, Bayarri, Tárrega, & Costell, 2011). Inulin, in particular high molecular weight inulin, increased stool frequency and can thus be used against constipation (Den Hond, Geypens, & Ghoos, 2000). It has been shown to improve stool frequency in formula fed newborns, creating a gut microbiota closer to that associated with breastfeeding (Closa-Monasterolo et al., 2013). Inulin was also able to relieve constipation in elderly patients, indicating that the stool promoting effect of inulin is present for all ages (Marteau et al., 2011).

2.3.2.2. Inflammatory bowel disease & colon cancer. Oral administration of inulin has been reported to achieve both local and systemic immune modulation. A review of the supporting evidence concluded that the local immunomodulatory effect is apparent, but systemic effects are less substantiated (Seifert & Watzl, 2007). The local effect is possibly indirect through the prebiotic action of inulin, stimulating growth of beneficial bacteria in the gut (Guarner, 2005; Kalyani Nair, Kharb, & Thompkinson, 2010). In a later in vitro study, it was found that inulin also possesses direct signaling capacity on human immune cells, mainly through the toll-like receptor 2 (Vogt et al., 2013). It is conceivable that these local effects could be beneficial to patients suffering from inflammatory bowel disease and irritable bowel syndrome (Leenen & Dieleman, 2007). Thus far, investigations with small numbers of patients have reported mixed results in this regard and there is thus a need for further investigation of this potential application of inulin (Kelly, 2009; Roberfroid, 2007).

Inulin was able to reduce chemically induced pre-neoplastic lesions or tumors in the colon of mice and rats (Pool-Zobel, 2005; Verma & Shukla, 2013). Long chain inulins were more potent than smaller chain inulins in this sense. This reduction was associated with the gut flora-mediated fermentation of inulin (Pool-Zobel, 2005). In a recent study in rats, inulin showed to have a bigger prophylactic potential to colon carcinogenesis compared to lactulose (Verma & Shukla, 2013). Inulin reduced cytotoxicity and genotoxicity in vitro in human colon adenocarcinoma cells (Adebola, Corcoran, & Morgan, 2013). Again these results seem promising, but further clinical research is required to see if these results can be reproduced in vivo in humans.

3. Overview

In this review the pharmaceutical applications of inulin, an oligosaccharide being increasingly used in food, pharma and other fields, have been reviewed. The most widely established use of inulin in pharma is as a diagnostic to determine kidney function. Inulin's chemical structure makes that it is not metabolized by the body and excreted completely via glomerular filtration. Gut microbiota, however, are able to metabolize inulin. This makes inulin suitable for colon specific drug delivery and as a prebiotic. There is some evidence that inulin's prebiotic characteristics also lead to health benefits, particularly for patients with inflammatory bowel disease or in prevention of colon cancer. These claims, however, need further evidence.

Solid dispersions of amorphous inulin can be used to improve the dissolution behavior of lipophilic drugs. Amorphous inulin was shown to be a suitable stabilizer for membranes and proteins, protecting them against drought and elevated temperatures. Inulin has not yet been used in commercial formulations for this purpose but as the number of new biopharmaceuticals keeps increasing, it could be an interesting new stabilizer. In addition, insoluble isoforms of inulin have an adjuvant effect on the immune response achieved with several vaccines. Inulin could therefore serve two purposes as an excipient for vaccines, achieving both stabilization and an increased effectivity. Overall the uses of inulin are already diverse. Additionally more and more research is being done with chemically modified inulins, making it likely that more applications will be found for this flexible oligosaccharide.

Acknowledgements

This research was jointly financed by Royal FrieslandCampina, the European Union, European Regional Development Fund and The Ministry of Economic Affairs, Agriculture and Innovation, Peaks in the Delta, the Municipality of Groningen, the Provinces of Groningen, Fryslân and Drenthe as well as the Dutch Carbohydrate Competence Center.

References

Adebola, O., Corcoran, O., & Morgan, W. A. (2013). Protective effects of prebiotics inulin and lactulose from cytotoxicity and genotoxicity in human colon adenocarcinoma cells. *Food Research International*, *52*(1), 269–274. http://dx.doi.org/10.1016/j.foodres.2013.03.024

Akhgari, A., Farahmand, F., Afrasiabi Garekani, H., Sadeghi, F., & Vandamme, T. F. (2006). Permeability and swelling studies on free films containing inulin in combination with different polymethacrylates aimed for colonic drug delivery. *European Journal of Pharmaceutical Sciences*, *28*(4), 307–314. http://dx.doi.org/10.1016/j.ejps.2006.03.005

Amorij, J.-P., Meulenaar, J., Hinrichs, W. L. J., Stegmann, T., Huckriede, A., Coenen, F., et al. (2007). Rational design of an influenza subunit vaccine powder with sugar glass technology: Preventing conformational changes of haemagglutinin during freezing and freeze-drying. *Vaccine*, *25*(35), 6447–6457. http://dx.doi.org/10.1016/j.vaccine.2007.06.054

Amorij, J.-P., Saluja, V., Petersen, A. H., Hinrichs, W. L. J., Huckriede, A., & Frijlink, H. W. (2007). Pulmonary delivery of an inulin-stabilized influenza subunit vaccine prepared by spray-freeze drying induces systemic, mucosal humoral as well as cell-mediated immune responses in BALB/c mice. *Vaccine*, *25*(52), 8707–8717. http://dx.doi.org/10.1016/j.vaccine.2007.10.035

Apolinário, A. C., de Lima Damasceno, B. P. G., de Macêdo Beltrão, N. E., Pessoa, A., Converti, A., & da Silva, J. A. (2014). Inulin-type fructans: A review on different aspects of biochemical and pharmaceutical technology. *Carbohydrate Polymers*, *101*, 368–378. http://dx.doi.org/10.1016/j.carbpol.2013.09.081

Audouy, S. A. L., van der Schaaf, G., Hinrichs, W. L. J., Frijlink, H. W., Wilschut, J., & Huckriede, A. (2011). Development of a dried influenza whole inactivated virus vaccine for pulmonary immunization. *Vaccine*, *29*(26), 4345–4352. http://dx.doi.org/10.1016/j.vaccine.2011.04.029

Ayen, W. Y., & Kumar, N. (2012). A systematic study on lyophilization process of polymersomes for long-term storage using doxorubicin-loaded (PEG)3–PLA nanopolymersomes. *European Journal of Pharmaceutical Sciences*, *46*(5), 405–414. http://dx.doi.org/10.1016/j.ejps.2012.03.005

Barclay, T., Ginic-Markovic, M., Cooper, P., & Petrovsky, N. (2010). Inulin: A versatile polysaccharide with multiple pharmaceutical and food chemical uses. *Journal of Excipients and Food Chemicals*, *1*, 27–50.

Castelli, F., Sarpietro, M. G., Micieli, D., Ottimo, S., Pitarresi, G., Tripodo, G., et al. (2008). Differential scanning calorimetry study on drug release from an inulin-based hydrogel and its interaction with a biomembrane model: pH and loading effect. *European Journal of Pharmaceutical Sciences*, *35*(1–2), 76–85. http://dx.doi.org/10.1016/j.ejps.2008.06.005

Chen, S., Guo, D., Guo, B., Liu, J., Shen, Y., Xu, X., et al. (2012). Investigation on formulation and preparation of adenovirus encoding human endostatin lyophilized powders. *International Journal of Pharmaceutics*, *427*(2), 145–152. http://dx.doi.org/10.1016/j.ijpharm.2011.12.056

Closa-Monasterolo, R., Gispert-Llaurado, M., Luque, V., Ferre, N., Rubio-Torrents, C., Zaragoza-Jordana, M., et al. (2013). Safety and efficacy of inulin and

oligofructose supplementation in infant formula: Results from a randomized clinical trial. *Clinical Nutrition*, *32*(6), 918–927. http://dx.doi.org/10.1016/j.clnu.2013.02.009

Colaço, C., Sen, S., Thangavelu, M., Pinder, S., & Roser, B. (1992). Extraordinary stability of enzymes dried in trehalose: Simplified molecular biology. *Biotechnology (Nature Publishing Company)*, *10*(9), 1007–1011. http://dx.doi.org/10.1038/nbt0992-1007

Cooper, P. D., & Carter, M. (1986a). Anti-complementary action of polymorphic "solubility forms" of particulate inulin. *Molecular Immunology*, *23*, 895–901.

Cooper, P. D., & Carter, M. (1986b). The anti-melanoma activity of inulin in mice. *Molecular Immunology*, *23*(8), 903–908. http://dx.doi.org/10.1016/0161-5890(86)90076-3

Cooper, P. D., & Petrovsky, N. (2011). Delta inulin: A novel, immunologically active, stable packing structure comprising-D-[2-> 1] poly(fructo-furanosyl)-D-glucose polymers. *Glycobiology*, *21*(5), 595–606. http://dx.doi.org/10.1093/glycob/cwq201

Cooper, P. D., & Steele, E. J. (1988). The adjuvanticity of gamma inulin. *Immunology and Cell Biology*, *66*(5–6), 345–352. http://dx.doi.org/10.1038/icb.1988.45

Crowe, J. H., Carpenter, J. F., & Crowe, L. M. (1998). The role of vitrification in anhydrobiosis. *Annual Review of Physiology*, *60*(1), 73–103. http://dx.doi.org/10.1146/annurev.physiol.60.1.73

Crowe, J. H., Hoekstra, F. A., & Crowe, L. M. (1992). Anhydrobiosis. *Annual Review of Physiology*, *54*(1), 579–599. http://dx.doi.org/10.1146/annurev.ph.54.030192.003051

Crowe, J. H., Leslie, S. B., & Crowe, L. M. (1994). Is vitrification sufficient to preserve liposomes during freeze-drying? *Cryobiology*, *31*(4), 355–366. http://dx.doi.org/10.1006/cryo.1994.1043

Crowe, J. H., Oliver, A. E., Hoekstra, F. A., & Crowe, L. M. (1997). Stabilization of dry membranes by mixtures of hydroxyethyl starch and glucose: The role of vitrification. *Cryobiology*, *35*(1), 20–30. http://dx.doi.org/10.1006/cryo.1997.2020

Crum, M., Elkordy, A. A., Zarara, M., & Elkordy, E. A. (2013). In situ lyophilisation of nifedipine directly in hard gelatine capsules. *Pharmaceutical Development and Technology*, *18*(6), 1379–1390. http://dx.doi.org/10.3109/10837450.2012.723718

De Jonge, J., Amorij, J.-P., Hinrichs, W. L. J., Wilschut, J., Huckriede, A., & Frijlink, H. W. (2007). Inulin sugar glasses preserve the structural integrity and biological activity of influenza virosomes during freeze-drying and storage. *European Journal of Pharmaceutical Sciences*, *32*(1), 33–44. http://dx.doi.org/10.1016/j.ejps.2007.05.112

Delanaye, P., Thibaudin, L., Souvignet, M., Maillard, N., Alamartine, E., Rozet, E., et al. (2012). Comparison of acid and enzymatic methods for inulin dosage: Analytical performances and impact on glomerular filtration rate evaluation. *Clinica Chimica Acta*, *413*(5–6), 556–560. http://dx.doi.org/10.1016/j.cca.2011.11.024

Den Hond, E., Geypens, B., & Ghoos, Y. (2000). Effect of high performance chicory inulin on constipation. *Nutrition Research*, *20*(5), 731–736. http://dx.doi.org/10.1016/S0271-5317(00)00162-7

Eissens, A. C., Bolhuis, G. K., Hinrichs, W. L. J., & Frijlink, H. W. (2002). Inulin as filler-binder for tablets prepared by direct compaction. *European Journal of Pharmaceutical Sciences*, *15*(1), 31–38. http://dx.doi.org/10.1016/S0928-0987(01)00201-9

Eriksson, H. J. C., Hinrichs, W. L. J., van Veen, B., Somsen, G. W., de Jong, G. J., & Frijlink, H. W. (2002). Investigations into the stabilisation of drugs by sugar glasses: I. Tablets prepared from stabilised alkaline phosphatase. *International Journal of Pharmaceutics*, *249*(1–2), 59–70. Retrieved from ⟨http://www.ncbi.nlm.nih.gov/pubmed/12433434⟩.

Fares, M. M., Salem, M. S., & Khanfar, M. (2011). Inulin and poly(acrylic acid) grafted inulin for dissolution enhancement and preliminary controlled release of poorly water-soluble irbesartan drug. *International Journal of Pharmaceutics*, *410*(1–2), 206–211. http://dx.doi.org/10.1016/j.ijpharm.2011.03.029

Feher, J. (2012). Tubular reabsorption and secretion. In *Quantitative human physiology*. Boston: Elsevier. http://dx.doi.org/10.1016/B978-0-12-382163-8.00072-4

Flamm, G., Glinsmann, W., Kritchevsky, D., Prosky, L., & Roberfroid, M. (2001). Inulin and oligofructose as dietary fiber: A review of the evidence. *Critical Reviews in Food Science and Nutrition*, *41*(5), 353–362. http://dx.doi.org/10.1080/20014091091841

Flint, H. J., Scott, K. P., Louis, P., & Duncan, S. H. (2012). The role of the gut microbiota in nutrition and health. *Nature Reviews Gastroenterology & Hepatology*, *9*(10), 577–589. http://dx.doi.org/10.1038/nrgastro.2012.156

Fluckiger, F. A., & Hanbury, D. (1879). *Pharmacographia: A history of the principal drugs of vegetable origin met with in Great Britain and British India*. London: Macmillan and Co.

Franck, A. (2002). Technological functionality of inulin and oligofructose. *British Journal of Nutrition*, *87*(S2), S287. http://dx.doi.org/10.1079/BJN/2002550

Gordon, D., Kelley, P., Heinzel, S., Cooper, P., & Petrovsky, N. (2014). Immunogenicity and safety of Advax™, a novel polysaccharide adjuvant based on delta inulin, when formulated with hepatitis B surface antigen: A randomized controlled Phase 1 study. *Vaccine*, *32*(48), 6469–6477. http://dx.doi.org/10.1016/j.vaccine.2014.09.034

Grasmeijer, N., Stankovic, M., de Waard, H., Frijlink, H. W., & Hinrichs, W. L. J. (2013). Unraveling protein stabilization mechanisms: Vitrification and water replacement in a glass transition temperature controlled system. *Biochimica et Biophysica Acta (BBA)—Proteins and Proteomics*, *1834*(4), 763–769. http://dx.doi.org/10.1016/j.bbapap.2013.01.020

Guarner, F. (2005). Inulin and oligofructose: Impact on intestinal diseases and disorders. *British Journal of Nutrition*, *93*(S1), S61. http://dx.doi.org/10.1079/BJN20041345

Haj-Ahmad, R. R., Elkordy, A. A., Chaw, C. S., & Moore, A. (2013). Compare and contrast the effects of surfactants (Pluronic®F-127 and Cremophor®EL) and sugars (β-cyclodextrin and inulin) on properties of spray dried and crystallised lysozyme. *European Journal of Pharmaceutical Sciences*, *49*(4), 519–534. http://dx.doi.org/10.1016/j.ejps.2013.05.004

Hincha, D. K., Hellwege, E. M., Heyer, A. G., & Crowe, J. H. (2000). Plant fructans stabilize phosphatidylcholine liposomes during freeze-drying. *European Journal of Biochemistry*, *267*(2), 535–540. http://dx.doi.org/10.1046/j.1432-1327.2000.01028.x

Hincha, D. K., Zuther, E., Hellwege, E. M., & Heyer, A. G. (2002). Specific effects of fructo- and gluco-oligosaccharides in the preservation of liposomes during drying. *Glycobiology*, *12*(2), 103–110. http://dx.doi.org/10.1093/glycob/12.2.103

Hincha, D. K., Zuther, E., & Heyer, A. G. (2003). The preservation of liposomes by raffinose family oligosaccharides during drying is mediated by effects on fusion and lipid phase transitions. *Biochimica et Biophysica Acta (BBA)—Biomembranes*, *1612*(2), 172–177. http://dx.doi.org/10.1016/S0005-2736(03)00116-0

Hinrichs, W. L. J., Manceñido, F. A., Sanders, N. N., Braeckmans, K., De Smedt, S. C., Demeester, J., et al. (2006). The choice of a suitable oligosaccharide to prevent aggregation of PEGylated nanoparticles during freeze thawing and freeze drying. *International Journal of Pharmaceutics*, *311*(1–2), 237–244. http://dx.doi.org/10.1016/j.ijpharm.2005.12.032

Hinrichs, W. L. J., Prinsen, M. G., & Frijlink, H. W. (2001). Inulin glasses for the stabilization of therapeutic proteins. *International Journal of Pharmaceutics*, *215*(1–2), 163–174. http://dx.doi.org/10.1016/S0378-5173(00)00677-3

Hinrichs, W. L. J., Sanders, N. N., De Smedt, S. C., Demeester, J., & Frijlink, H. W. (2005). Inulin is a promising cryo- and lyoprotectant for PEGylated lipoplexes. *Journal of Controlled Release*, *103*(2), 465–479. http://dx.doi.org/10.1016/j.jconrel.2004.12.011 (Official Journal of the Controlled Release Society)

Honda-Okubo, Y., Kolpe, A., Li, L., & Petrovsky, N. (2014). A single immunization with inactivated H1N1 influenza vaccine formulated with delta inulin adjuvant (Advax™) overcomes pregnancy-associated immune suppression and enhances passive neonatal protection. *Vaccine*, *32*(36), 4651–4659. http://dx.doi.org/10.1016/j.vaccine.2014.06.057

Honda-Okubo, Y., Saade, F., & Petrovsky, N. (2012). Advax™, a polysaccharide adjuvant derived from delta inulin, provides improved influenza vaccine protection through broad-based enhancement of adaptive immune responses. *Vaccine*, *30*(36), 5373–5381. http://dx.doi.org/10.1016/j.vaccine.2012.06.021

Jain, A. K., Sood, V., Bora, M., Vasita, R., & Katti, D. S. (2014). Electrosprayed inulin microparticles for microbiota triggered targeting of colon. *Carbohydrate Polymers*, *112*, 225–234. http://dx.doi.org/10.1016/j.carbpol.2014.05.087

Janssens, S., Van Humbeeck, J., & Van den Mooter, G. (2008). Evaluation of the formulation of solid dispersions by co-spray drying itraconazole with Inutec SP1, a polymeric surfactant, in combination with PVPVA 64. *European Journal of Pharmaceutics and Biopharmaceutics*, *70*(2), 500–505. http://dx.doi.org/10.1016/j.ejpb.2008.05.025

Kalyani Nair, K., Kharb, S., & Thompkinson, D. K. (2010). Inulin dietary fiber with functional and health attributes—A review. *Food Reviews International*, *26*(2), 189–203. http://dx.doi.org/10.1080/87559121003590664

Kelly, G. (2008). Inulin-type prebiotics—A review: Part 1. *Alternative Medicine Review: A Journal of Clinical Therapeutic*, *13*(4), 315–329.

Kelly, G. (2009). Inulin-type prebiotics: A review (Part 2). *Alternative Medicine Review*, *4*(March (1)), 36–55.

Kolida, S., Tuohy, K., & Gibson, G. R. (2002). Prebiotic effects of inulin and oligofructose. *British Journal of Nutrition*, *87*(S2), S193. http://dx.doi.org/10.1079/BJN/2002537

Kruger, C. L. (2002). *Generally recognised as safe (GRAS) notification for Frutafit®*. Arlington, VA: Food and Drug Administration.

Kumar, S., & Tummala, H. (2013). Development of soluble inulin microparticles as a potent and safe vaccine adjuvant and delivery system. *Molecular Pharmaceutics*, *10*(5), 1845–1853. http://dx.doi.org/10.1021/mp3006374

Layton, R. C., Petrovsky, N., Gigliotti, A. P., Pollock, Z., Knight, J., Donart, N., et al. (2011). Delta inulin polysaccharide adjuvant enhances the ability of split-virion H5N1 vaccine to protect against lethal challenge in ferrets. *Vaccine*, *29*(37), 6242–6251. http://dx.doi.org/10.1016/j.vaccine.2011.06.078

Leenen, C. H. M., & Dieleman, L. A. (2007). Inulin and oligofructose in chronic inflammatory bowel disease. *The Journal of Nutrition*, *137*(11 Suppl), 2572S–2575S, 137/11/2572S.

Livingston, D., Hincha, D., & Heyer, A. (2007). The relationship of fructan to abiotic stress tolerance in plants. In S. Norio, B. Noureddine, & O. Shuichi (Eds.), *Recent advances in fructooligosaccharides research* (vol. 661) (pp. 181–199).

López-Molina, D., Chazarra, S., How, C. W., Pruidze, N., Navarro-Perán, E., & García-Cánovas, F. (2015). Cinnamate of inulin as a vehicle for delivery of colonic drugs. *International Journal of Pharmaceutics*, *479*(1), 96–102. http://dx.doi.org/10.1016/j.ijpharm.2014.12.064

Maris, B., Verheyden, L., Van Reeth, K., Samyn, C., Augustijns, P., Kinget, R., et al. (2001). Synthesis and characterisation of inulin-azo hydrogels designed for colon targeting. *International Journal of Pharmaceutics*, *213*(1–2), 143–152. http://dx.doi.org/10.1016/S0378-5173(00)00654-2

Marteau, P., Jacobs, H., Cazaubiel, M., Signoret, C., Prevel, J.-M., & Housez, B. (2011). Effects of chicory inulin in constipated elderly people: A double-blind controlled trial. *International Journal of Food Sciences and Nutrition*, *62*(2), 164–170. http://dx.doi.org/10.3109/09637486.2010.527323

Matvieieva, N. A., Luchakivska, Y. S., Kvasko, O. Y., Afanasieva, K. S., Zazhyska, M. O., & Kuchuk, M. V. (2013). In vitro antarctic mosses: Fructan synthesis under abiotic stress conditions. *Journal of International Scientific Publications: Agriculture & Food*, *1*(2), 22–25. Retrieved from ⟨http://www.scientific-publications.net/download/agriculture-and-food-2013-2.pdf#page=22⟩.

Mensink, M. A., Frijlink, H. W., van der Voort Maarschalk, K., & Hinrichs, W. L. J. (2015). Inulin, a flexible oligosaccharide. I: Review of its physicochemical characteristics. *Carbohydrate Polymers*, *130*, 405–419. http://dx.doi.org/10.1016/j.carbpol.2015.05.026

Meyer, D., Bayarri, S., Tárrega, A., & Costell, E. (2011). Inulin as texture modifier in dairy products. *Food Hydrocolloids*, *25*(8), 1881–1890. http://dx.doi.org/10.1016/j.foodhyd.2011.04.012

Murugappan, S., Frijlink, H. W., Petrovsky, N., & Hinrichs, W. L. J. (2015). Enhanced pulmonary immunization with aerosolized inactivated influenza vaccine containing delta inulin adjuvant. *European Journal of Pharmaceutical Sciences*, *66*, 118–122. http://dx.doi.org/10.1016/j.ejps.2014.10.008

Orlando, R., Floreani, M., Padrini, R., & Palatini, P. (2002). Determination of inulin clearance by bolus intravenous injection in healthy subjects and ascitic patients: Equivalence of systemic and renal clearances as glomerular filtration markers. *British Journal of Clinical Pharmacology*, *46*(6), 605–609. http://dx.doi.org/10.1046/j.1365-2125.1998.00824.x

Pfeil, W., & Privalov, P. L. (1976). Thermodynamic investigations of proteins. I. Standard functions for proteins with lysozyme as an example. *Biophysical Chemistry*, *4*(1), 23–32. http://dx.doi.org/10.1016/0301-4622(76)80003-8

Pool-Zobel, B. L. (2005). Inulin-type fructans and reduction in colon cancer risk: Review of experimental and human data. *British Journal of Nutrition*, *93*(S1), S73. http://dx.doi.org/10.1079/BJN20041349

Poulain, N., Dez, I., Perrio, C., Lasne, M.-C., Prud'homme, M.-P., & Nakache, E. (2003). Microspheres based on inulin for the controlled release of serine protease inhibitors: Preparation, characterization and in vitro release. *Journal of Controlled Release*, *92*(1–2), 27–38. http://dx.doi.org/10.1016/S0168-3659(03)00251-7

Roberfroid, M. B. (2007). Inulin-type fructans: Functional food ingredients. *The Journal of Nutrition*, *137*(11 Suppl.), 2493S–2502S. Retrieved from ⟨http://jn.nutrition.org/content/137/11/2493S.short⟩.

Roberfroid, M. B., & Delzenne, N. M. (1998). Dietary fructans. *Annual Review of Nutrition*, *18*(1), 117–143. http://dx.doi.org/10.1146/annurev.nutr.18.1.117

Rodríguez Furlán, L. T., Lecot, J., Pérez Padilla, A., Campderrós, M. E., & Zaritzky, N. (2011). Effect of saccharides on glass transition temperatures of frozen and freeze dried bovine plasma protein. *Journal of Food Engineering*, *106*(1), 74–79. http://dx.doi.org/10.1016/j.jfoodeng.2011.04.010

Rodríguez Furlán, L. T., Padilla, A. P., & Campderrós, M. E. (2010). Inulin like lyoprotectant of bovine plasma proteins concentrated by ultrafiltration. *Food Research International*, *43*(3), 788–796. http://dx.doi.org/10.1016/j.foodres.2009.11.015

Rodriguez Furlán, L. T., Pérez Padilla, A., & Campderros, M. (2011). Development of a functional beverage formulation with high protein content, inulin and stevia. *International Journal of Food Engineering*, *7*(3), 1–16. http://dx.doi.org/10.2202/1556-3758.2014

Saade, F., Honda-Okubo, Y., Trec, S., & Petrovsky, N. (2013). A novel hepatitis B vaccine containing Advax™, a polysaccharide adjuvant derived from delta inulin, induces robust humoral and cellular immunity with minimal reactogenicity in preclinical testing. *Vaccine*, *31*(15), 1999–2007. http://dx.doi.org/10.1016/j.vaccine.2012.12.077

Saluja, V., Amorij, J.-P., Kapteyn, J. C., de Boer, A. H., Frijlink, H. W., & Hinrichs, W. L. J. (2010). A comparison between spray drying and spray freeze drying to produce an influenza subunit vaccine powder for inhalation. *Journal of Controlled Release*, *144*(2), 127–133. http://dx.doi.org/10.1016/j.jconrel.2010.02.025

Sardo, C., Farra, R., Licciardi, M., Dapas, B., Scialabba, C., Giammona, G., et al. (2015). Development of a simple, biocompatible and cost-effective inulin—Diethylenetriamine based siRNA delivery system. *European Journal of Pharmaceutical Sciences*, *75*, 60–71. http://dx.doi.org/10.1016/j.ejps.2015.03.021

Seifert, S., & Watzl, B. (2007). Inulin and oligofructose: Review of experimental data on immune modulation. *The Journal of Nutrition*, *137*(11 Suppl), 2563S–2567S, 137/11/2563S.

Sharma, P., & Pathak, K. (2013). Inulin-based tablet in capsule device for variable multipulse delivery of aceclofenac: Optimization and in vivo roentgenography. *AAPS PharmSciTech*, *14*(2), 736–747. http://dx.doi.org/10.1208/s12249-013-9959-8

Silva, D. G., Cooper, P. D., & Petrovsky, N. (2004). Inulin-derived adjuvants efficiently promote both Th1 and Th2 immune responses. *Immunology and Cell Biology*, *82*(6), 611–616. http://dx.doi.org/10.1111/j.1440-1711.2004.01290.[01290.x]x

Srinarong, P., Am, S. H., Visser, M. R., Hinrichs, W. L. J., Ketolainen, J., & Frijlink, H. W. (2011). Surface-active derivative of inulin (nutec R SP1) is a superior carrier for solid dispersions with a high drug load. *Journal of Pharmaceutical Sciences*, *100*(6) http://dx.doi.org/10.1002/jps.22471

Srinarong, P., Faber, J. H., Visser, M. R., Hinrichs, W. L. J., & Frijlink, H. W. (2009). Strongly enhanced dissolution rate of fenofibrate solid dispersion tablets by incorporation of superdisintegrants. *European Journal of Pharmaceutics and Biopharmaceutics*, *73*(1), 154–161. http://dx.doi.org/10.1016/j.ejpb.2009.05.006

Stanković, M., de Waard, H., Steendam, R., Hiemstra, C., Zuidema, J., Frijlink, H. W., et al. (2013). Low temperature extruded implants based on novel hydrophilic multiblock copolymer for long-term protein delivery. *European Journal of Pharmaceutical Sciences*, *49*(4), 578–587. http://dx.doi.org/10.1016/j.ejps.2013.05.011

Stubbe, B., Maris, B., Van den Mooter, G., De Smedt, S. C., & Demeester, J. (2001). The in vitro evaluation of "azo containing polysaccharide gels" for colon delivery. *Journal of Controlled Release*, *75*(1–2), 103–114. http://dx.doi.org/10.1016/S0168-3659(01)00367-4

The Editors of Encyclopaedia Brittanica. (2015). *Inulin clearance*. Retrieved March 27, 2015, from ⟨http://www.britannica.com/EBchecked/topic/292226/inulin-clearance⟩.

Tonnis, W. F., Lexmond, A. J., Frijlink, H. W., de Boer, A. H., & Hinrichs, W. L. J. (2013). Devices and formulations for pulmonary vaccination. *Expert Opinion on Drug Delivery*, *10*(10), 1383–1397. http://dx.doi.org/10.1517/17425247.2013.810622

Tonnis, W. F., Mensink, M. A., de Jager, A., van der Voort Maarschalk, K., Frijlink, H. W., & Hinrichs, W. L. J. (2015). Size and molecular flexibility of sugars determine the storage stability of freeze-dried proteins. *Molecular Pharmaceutics*, *12*(3), 684–694. http://dx.doi.org/10.1021/mp500423z

Tripodo, G., Pitarresi, G., Cavallaro, G., Palumbo, F. S., & Giammona, G. (2009). Controlled release of IgG by novel UV induced polysaccharide/poly(amino acid) hydrogels. *Macromolecular Bioscience*, *9*(4), 393–401. http://dx.doi.org/10.1002/mabi.200800181

Tripodo, G., Pitarresi, G., Palumbo, F. S., Craparo, E. F., & Giammona, G. (2005). UV-photocrosslinking of inulin derivatives to produce hydrogels for drug delivery application. *Macromolecular Bioscience*, *5*(11), 1074–1084. http://dx.doi.org/10.1002/mabi.200500134

Van den Mooter, G., Vervoort, L., & Kinget, R. (2003). Characterization of methacrylated inulin hydrogels designed for colon targeting: In vitro release of BSA. *Pharmaceutical Research*, *20*(2), 303–307. http://dx.doi.org/10.1023/A:1022295725841

Van Drooge, D.-J., Hinrichs, W. L. J., Dickhoff, B. H. J., Elli, M. N. A., Visser, M. R., Zijlstra, G. S., & Frijlink, H. W. (2005). Spray freeze drying to produce a stable Δ^9-tetrahydrocannabinol containing inulin-based solid dispersion powder suitable for inhalation. *European Journal of Pharmaceutical Sciences*, *26*(2), 231–240. http://dx.doi.org/10.1016/j.ejps.2005.06.007

Van Drooge, D.-J., Hinrichs, W. L. J., & Frijlink, H. W. (2004). Anomalous dissolution behaviour of tablets prepared from sugar glass-based solid dispersions. *Journal of Controlled Release*, *97*(3), 441–452. http://dx.doi.org/10.1016/j.jconrel.2004.03.018 (Official Journal of the Controlled Release Society)

Van Drooge, D.-J., Hinrichs, W. L. J., Wegman, K. A. M., Visser, M. R., Eissens, A. C., & Frijlink, H. W. (2004). Solid dispersions based on inulin for the stabilisation and formulation of Δ^9-tetrahydrocannabinol. *European Journal of Pharmaceutical Sciences*, *21*(4), 511–518. http://dx.doi.org/10.1016/j.ejps.2003.11.014

Vereyken, I. J., Albert van Kuik, J., Evers, T. H., Rijken, P. J., & de Kruijff, B. (2003). Structural requirements of the fructan–lipid interaction. *Biophysical Journal*, *84*(5), 3147–3154. http://dx.doi.org/10.1016/S0006-3495(03)70039-3

Vereyken, I. J., Chupin, V., Demel, R. A., Smeekens, S. C., & De Kruijff, B. (2001). Fructans insert between the headgroups of phospholipids. *Biochimica et Biophysica Acta (BBA)—Biomembranes*, *1510*(1–2), 307–320. http://dx.doi.org/10.1016/S0005-2736(00)00363-1

Vereyken, I. J., Chupin, V., Hoekstra, F. A., Smeekens, S. C. M., & de Kruijff, B. (2003). The effect of fructan on membrane lipid organization and dynamics in the dry state. *Biophysical Journal*, *84*(6), 3759–3766. http://dx.doi.org/10.1016/S0006-3495(03)75104-2

Vereyken, I. J., Chupin, V., Islamov, A., Kuklin, A., Hincha, D. K., & de Kruijff, B. (2003). The effect of fructan on the phospholipid organization in the dry state. *Biophysical Journal*, *85*(5), 3058–3065. http://dx.doi.org/10.1016/S0006-3495(03)74724-9

Verma, A., & Shukla, G. (2013). Administration of prebiotic inulin suppresses 1,2 dimethylhydrazine dihydrochloride induced procarcinogenic biomarkers fecal enzymes and preneoplastic lesions in early colon carcinogenesis in Sprague Dawley rats. *Journal of Functional Foods*, *5*(2), 991–996. http://dx.doi.org/10.1016/j.jff.2013.02.006

Vervoort, L., Rombaut, P., Van den Mooter, G., Augustijns, P., & Kinget, R. (1998). Inulin hydrogels. II. In vitro degradation study. *International Journal of Pharmaceutics*, *172*(1–2), 137–145. http://dx.doi.org/10.1016/S0378-5173(98)00201-4

Vervoort, L., Van den Mooter, G., Augustijns, P., Busson, R., Toppet, S., & Kinget, R. (1997). Inulin hydrogels as carriers for colonic drug targeting: I. Synthesis and characterization of methacrylated inulin and hydrogel formation. *Pharmaceutical Research*, *14*(12), 1730–1737. http://dx.doi.org/10.1023/A:1012179813102

Visser, M. R., Baert, L., Klooster, G. van't, Schueller, L., Geldof, M., Vanwelkenhuysen, I., et al. (2010). Inulin solid dispersion technology to improve the absorption of the BCS Class IV drug TMC240. *European Journal of Pharmaceutics and Biopharmaceutics*, *74*(2), 233–238. http://dx.doi.org/10.1016/j.ejpb.2009.10.004

Vogt, L., Ramasamy, U., Meyer, D., Pullens, G., Venema, K., Faas, M. M., et al. (2013). Immune modulation by different types of $\beta 2 \rightarrow 1$-fructans is toll-like receptor dependent. *PLoS ONE*, *8*(7), e68367. http://dx.doi.org/10.1371/journal.pone.0068367

Wada, T., Sugatani, J., Terada, E., Ohguchi, M., & Miwa, M. (2005). Physicochemical characterization and biological effects of inulin enzymatically synthesized from sucrose. *Journal of Agricultural and Food Chemistry*, *53*, 1246–1253.

Wahjudi, M., Murugappan, S., van Merkerk, R., Eissens, A. C., Visser, M. R., Hinrichs, W. L. J., et al. (2013). Development of a dry, stable and inhalable

acyl–homoserine–lactone–acylase powder formulation for the treatment of pulmonary *Pseudomonas aeruginosa* infections. *European Journal of Pharmaceutical Sciences*, *48*(4–5), 637–643. http://dx.doi.org/10.1016/j.ejps.2012.12.015

Wu, X. Y., & Lee, P. I. (2000). Preparation and characterization of inulin ester microspheres as drug carriers. *Journal of Applied Polymer Science*, *77*(4), 833–840. http://dx.doi.org/10.1002/(SICI)1097-4628(20000725)77:4<833::AID-APP17>3.0.CO;2-4

Zhang, L., Li, Y., Wang, C., Li, G., Zhao, Y., & Yang, Y. (2014). Synthesis of methylprednisolone loaded ibuprofen modified inulin based nanoparticles and their application for drug delivery. *Materials Science and Engineering: C*, *42*, 111–115. http://dx.doi.org/10.1016/j.msec.2014.05.025

Zijlstra, G. S., Ponsioen, B. J., Hummel, S. A., Sanders, N., Hinrichs, W. L. J., de Boer, A. H., et al. (2009). Formulation and process development of (recombinant human) deoxyribonuclease I as a powder for inhalation. *Pharmaceutical Development and Technology*, *14*(4), 358–368. http://dx.doi.org/10.1080/10837450802662820

Zijlstra, G. S., Wolting, J., Prop, J., Petersen, A. H., Hinrichs, W. L. J., Uges, D. R. A., et al. (2009). Efficacy of a new pulmonary cyclosporine A powder formulation for prevention of transplant rejection in rats. *The Journal of Heart and Lung Transplantation*, *28*(5), 486–492. http://dx.doi.org/10.1016/j.healun.2009.02.001